

Rectovaginal endometriosis – pain treatment options

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Academic dissertation

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ABSTRACT

Endometriosis is a common gynecological disease affecting women in their best reproductive years. It is divided into three forms depending on the location and depth of the disease; superficial peritoneal endometriosis, ovarian endometriosis and deep infiltrating endometriosis (DIE). DIE located in the rectovaginal septum is referred to as rectovaginal endometriosis (RVE). It causes severe pain via mechanisms that are partly unknown. Inflammation and increased innervation in endometriotic tissue appear to have a central role in the generation of these symptoms.

The first-line treatment of endometriosis, RVE included, is hormone therapy using combined oral contraceptives (COCs) or progestins. In cases of RVE, however, this is not always sufficient. In symptomatic patients not responding to hormonal therapies (HTs), radical surgery is needed – meaning excising all the visible endometriotic tissue from the pelvic and abdominal areas. Surgical treatment of RVE extending into colorectum may require bowel resection. The long-term effects of surgery for RVE have not been well studied. The ultimate aim of this study was to acquire information on the long-term outcome of RVE surgery.

Patient charts concerning RVE surgery (153 cases) between 2000–2004 were studied to investigate the outcome (Study I). In addition, we aimed to identify factors associated with bowel surgery; for adequate patient counseling and operative planning, patients who need colorectal surgery should be identified early on.

To study the long-term effects of surgery, patients operated upon in 2002–2004 were offered a follow-up visit to our clinic on average four years after the original operation. Altogether, 60 patients agreed to take part in this study (Study II). Symptom recurrence was evaluated by using symptom diaries.

We also studied charts on 164 patients who had undergone bowel resection in 2004–2012. The laparoscopic approach to bowel resection was of special interest (Study III), with emphasis on collecting information on complications.

Since increased densities of nerve fibers in endometriotic tissue may contribute to pain generation, we used immunohistochemistry to study their types and presence in samples obtained during surgery in 2000–2004. Altogether, 45 samples were eligible for this study (Study IV). We also studied the effects of HT on the density of nerve fibers.

Our results showed that even though clinical recurrence developed in 35% of cases, symptom recurrence was rare. Bowel resection was protective as regards recurrence, as were all the forms of hormone and surgical therapy that resulted in amenorrhea.

Laparoscopy offers a safe route for performing RVE surgery, and should be the primary approach even as regards colorectal resection in cases of RVE. We found that the rate of complications did not depend on the type of surgery (laparoscopy vs. laparotomy) but was more related to the experience of the surgeon.

Excessive innervation by sympathetic, parasympathetic and sensory nerve fibers was identified in the surgical RVE specimens. This innervation may be one of the explanations for the severe pain that these patients experience. The density of nerve fibers was reduced among those patients who were using either COCs or progestins.

In conclusion, in experienced hands, surgery for RVE results in long-term symptom relief. In order to avoid symptom recurrence, HTs with the aim of amenorrhea should be administered following surgical treatment. Reducing the densities of nerve fibers in endometriotic tissue may be a novel therapeutic mechanism of action of HTs. More studies on this important topic are warranted.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals:

- I Tarjanne S, Sjöberg J, Heikinheimo O; Rectovaginal endometriosis – characteristics of operative treatment and factors predicting bowel resection. *J Minim Invasive Gynecol* 2009 May-Jun;16(3):302–306.
- II Tarjanne S, Sjöberg J, Heikinheimo O; Radical excision of rectovaginal endometriosis results in high rate of pain relief – results of a long-term follow-up study. *Acta Obstet Gynecol Scand* 2010;89(1):71–77.
- III Tarjanne S, Heikinheimo O, Mentula M, Härkki P; Complications and long-term follow-up on colorectal resections in the treatment of deep infiltrating endometriosis extending to bowel wall. *Acta Obstet Gynecol Scand* 2015; 94(1):72–79.
- IV Tarjanne S, Ng CH, Manconi F, Arola J, Mentula M, Maneck B, Fraser IS, Heikinheimo O; Use of hormonal therapy is associated with reduced nerve fiber density in deep infiltrating, rectovaginal endometriosis. *Acta Obstet Gynecol Scand* 2015;94(7):693–700.

ABBREVIATIONS

A	androstenedione
AA	arachidonic acid
ACTH	adrenocorticotrophic hormone
Bcl-2	B cell lymphoma/leukemia-2
CHOL	cholesterol
CNS	central nervous system
COC	combined oral contraceptive
COX-2	cyclooxygenase-2
CPA	cyproterone acetate
CRH	corticotropin-releasing hormone
DEG	desogestrel
DIE	deep infiltrating endometriosis
DNG	dienogest
E ₁	estrone
E ₂	estradiol
EE ₂	ethinyl estradiol
EBAF	endometrial bleeding-associated factor
ER β	estrogen receptor β
ETOG	etonogestrel
FSH	follicle-stimulating hormone
GES	gestodene
GnRH	gonadotropin-releasing hormone
HGF	hepatocyte growth factor
HPA	hypothalamic-pituitary-adrenal

17 β HSD-1	17 β -hydroxysteroid dehydrogenase
HT	hormone therapy
LNG-IUS	levonorgestrel-releasing intrauterine system
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
NETA	norethisterone acetate
NG	norelgestromin
NGF	neural growth factor
NPY	Neuropeptide Y
NSAID	non-steroidal anti-inflammatory drug
PGE ₂	prostaglandin E ₂
PGP _{9.5}	Protein gene product 9.5
QoL	quality of life
RANTES	regulated on activation, normal T cell expressed and secreted
RCT	randomized controlled trial
RVE	rectovaginal endometriosis
SP	Substance P
SPRM	selective progesterone receptor modulator
TGF- β	tumor growth factor- β
TIMP	tissue inhibitor of metalloproteinase
TNF- α	tumor necrosis factor- α
TrkA	tyrosine kinase receptor A
US	ultrasonography
VEGF	vascular endothelial growth factor
VIP	Vasoactive intestinal peptide

INTRODUCTION

Endometriosis is a benign estrogen-dependent disease occurring in 6-10% of women of reproductive age (Lebovic et al., 2001, Giudice, 2010). It is frequently associated with severe pain symptoms such as dysmenorrhea, dyspareunia (pain during intercourse) or chronic pelvic pain. Endometriosis is also a common cause of infertility.

Three different forms of endometriosis have been recognized, depending primarily on the location of the disease – ovarian endometriosis, superficial peritoneal endometriosis and deep infiltrating endometriosis (DIE). DIE is commonly located in the utero-sacral ligaments, the cul-de-sac, or the upper posterior vaginal wall behind the cervix – in the last case, endometriosis is referred to as rectovaginal endometriosis (RVE). In 4-12% of RVE cases, DIE extends into the bowel wall (Wills et al., 2008).

Pain is the leading symptom in endometriosis. However, the origin of this pain remains enigmatic. Inflammation is considered the main factor in generating endometriosis-associated pain (Bergqvist et al., 2001, Sikora et al., 2012, Ahn et al., 2015). Estrogen is involved in promoting inflammation (Bulun 2009). In addition, endometriotic lesions – and RVE in particular – show a dense innervation by small unmyelinated nerve fibers (Tokushige et al., 2007). Hyperinnervation and inflammation seem to be closely connected as inflammation induces the release of several neurotrophic factors, which subsequently lead to nerve sprouting and pain (Maharajaa et al., 2014, McKinnon et al., 2015).

Various forms of hormone therapy (HT), in particular COCs and progestogens, are used as the first-line treatment for pain in RVE (Vercellini et al., 2009), since HT suppresses ovarian estrogen secretion, and reduces inflammation. However, other mechanisms of action may be involved as well; the decrease in retrograde menstruation, and the subsequent reduction of inflammatory agents of endometrial origin may also contribute to achieving pain relief. Interestingly, HT has also been shown to reduce the density of nerve fibers in lesions, adding possibly a completely novel explanation for its mechanism of action (Tokushige et al., 2009).

In RVE patients not responding to hormone therapy, the gold standard for treatment is surgery (Chapron et al., 2004). The surgical treatment of RVE is challenging, and preservation of fertility is demanded in most operations. A multidisciplinary approach is often needed, as in 35% of cases surgery involves bowel resection (Meuleman et al., 2011). During the past ten years, the route in performing bowel resection has changed from laparotomy to a laparoscopic approach (Ruffo et al., 2012). In large specialized centers, major complications occur in 9% of laparoscopic bowel resections

(Ruffo et al., 2012). It would be extremely important to identify patients requiring colorectal surgery in advance for adequate patient information and optimal planning of surgery.

Recurrences – return of lesions or pain – following endometriosis surgery present a major challenge. They occur in 25-30% of all endometriosis operations (Vercellini et al., 2009). Recurrence rates following RVE surgery vary between 3-35% (Fedele et al., 2004). Performing bowel resection may be protective against recurrence (Fedele et al. 2004). However, more studies are needed to identify factors associated with recurrence.

The aim of the present work was to study the long-term effects of surgery in the treatment of RVE, and in particular, to identify factors connected with recurrence. Also, an important aim was to identify patients requiring colorectal surgery, and to recognize factors associated with performing bowel resection. The feasibility of a laparoscopic approach to bowel resection among RVE patients needed evaluation as well, since the majority of these operations in Helsinki University Central Hospital are currently performed via laparoscopy. As nerve fibers may have a central role in generating pain, the densities of nerve fibers in endometriotic tissue removed during surgery were studied. In addition, the effect of HT on these nerve fiber densities was evaluated.

REVIEW OF THE LITERATURE

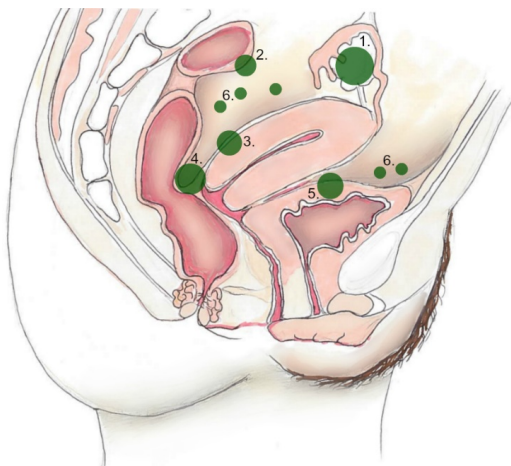
1. Definitions

Endometriosis is a chronic, benign, estrogen-dependent inflammatory disease defined as the presence of endometrial glands and stroma outside the uterus (Giudice & Kao, 2004). The most typical locations of endometriotic lesions are illustrated in Figure 1. Depending on the location, endometriosis is divided into superficial peritoneal endometriosis, ovarian endometriosis, or deep infiltrating endometriosis (DIE). Endometriotic implants penetrating the peritoneum deeper than 5 mm are defined as cases of DIE (Koninckx, 1991). In the literature, DIE is also referred to as adenomyosis externa because of the histological resemblance of these noduli (nodular aggregates of smooth muscle cells) to adenomyomas (Koninckx et al., 2012). The adenomyotic nodules typically involve the pouch of Douglas, the vesicouterine fold, the uterosacral ligaments, the upper vaginal wall behind the cervix, and/or the rectosigmoid colon.

Rectovaginal endometriosis (RVE) refers to DIE that extends from the posterior upper vaginal wall to the anterior rectal wall, and laterally to the uterosacral ligaments (Wolthuis et al., 2014). Recently, a precise definition of RVE has been suggested to be endometriosis of the retrocervical septum (Batt et al., 2014).

Figure 1. Typical locations of ectopic endometriotic lesions.

1. ovaries
2. bowel wall
3. sacrouterine ligaments
4. rectovaginal septum
5. bladder
6. peritoneum



2. Prevalence and significance of endometriosis

Endometriosis affects up to 6–10% of women of reproductive age (Giudice and Kao 2004, Gylfason et al., 2010). It is much more common among patients suffering from chronic pelvic pain and/or infertility, when its frequency can reach up to 35–50% (Giudice and Kao 2004). RVE is less common than ovarian or peritoneal endometriosis, affecting only 1% of women of fertile age (Slack et al., 2007, Koninckx et al., 2012). However, the presence of DIE is often underestimated; in a recent study among endometriosis patients undergoing surgery, the prevalence of DIE was 22%, and in 65% of DIE cases patients presented with a rectovaginal nodule (Knabben et al., 2015).

A prospective, multicenter study conducted in 10 European countries demonstrated that the average annual cost per patient with endometriosis was approximately 10 000 euros in 2008, including both health care and loss of productivity costs (Simoens et al., 2012). In a recent Danish study, endometriosis associated with impaired working ability (Hansen et al., 2013). Other inflammatory diseases (such as painful bladder syndrome, migraine or irritable bowel syndrome) as well as autoimmune diseases and asthma frequently coincide with the condition, adding to the economic and psychosocial burden of endometriosis (Kvaskoff et al., 2015)

3. Symptoms

Patients with endometriosis suffer from a variety of pain symptoms depending on the location of the disease (Table 1). Subfertility can be the first sign of endometriosis. The two most common symptoms in endometriosis are dysmenorrhea and abdominopelvic pain; approximately 70% of patients with endometriosis report either of these symptoms (Vercellini et al., 2007, Ballard et al., 2008). Curiously, no clear correlation exists between the extent of pelvic endometriosis in surgery, and the intensity of pain symptoms (Fauconnier and Chapron 2005). Severe preoperative symptoms, however, seem to be connected to the existence of deep lesions (Chapron et al., 2012). It has been estimated that over 95% of patients with DIE are symptomatic, and their quality of life can be compromised to a great extent due to these pain symptoms (for review see Koninckx et al., 2012).

In RVE, due to the anatomic location, the main complaints are dysmenorrhea, dyspareunia, pelvic pain and pain during defecation (Chapron et al., 2012). If endometriotic lesions infiltrate into the bowel wall, patients may present with bowel symptoms such as rectal bleeding, bloating, diarrhea, or constipation. DIE located in the urinary tract may be associated with dysuria or hematuria. Ureteral stenosis by ectopic endometriotic tissue may lead to impaired kidney function, usually unilaterally (Rozsnyai et al., 2011).

Table 1. Typical symptoms in endometriosis.

Symptoms
Dysmenorrhea
Non-cyclical pelvic pain
Coital pain
Dyschezia
Dysuria
Subfertility

4. Pathophysiology of endometriosis and pain

No consensus exists concerning the mechanisms underlying the generation of endometriotic implants. The most accepted theory is that of Sampson, published as early as in 1927. According to this theory, endometriosis results from backward (retrograde) menstrual flow from the uterine cavity through the fallopian tubes to the peritoneal surfaces (Sampson, 1927). Thereafter, menstrual fragments implant and persist through a variety of inflammatory and angiogenic processes (for review see Bulun 2009) (Figure 2).

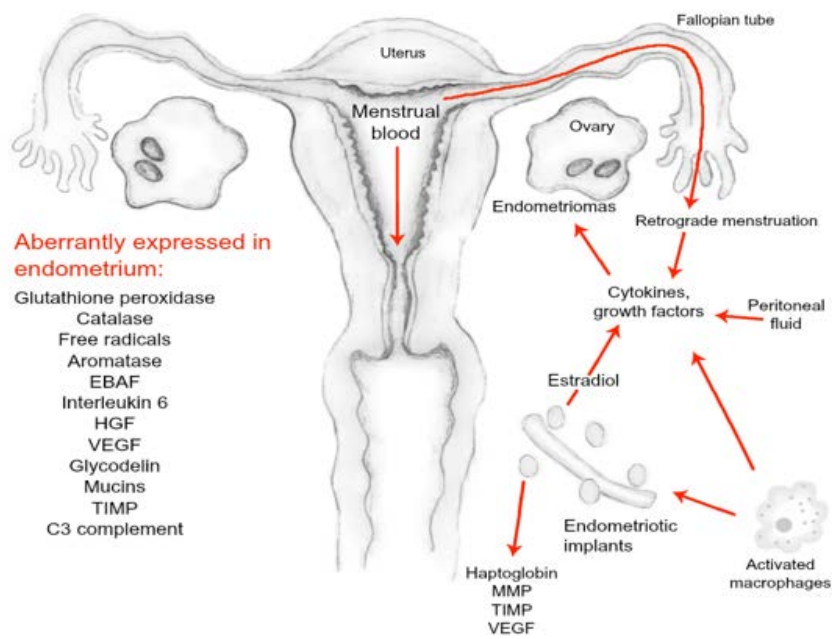
Altered immunology – involving dysfunctional macrophages and other immune cells – seems to have a central role in the development of endometriosis (Lebovic et al., 2001, Vercellini et al., 2014). Macrophages synthesize and secrete prostaglandins, cytokines, growth factors and angiogenic factors into the peritoneal fluid, these including interleukins, TNF- α , TGF- β , VEGF, COX-2, and the neurotrophin NGF (Giudice and Kao 2004, Sikora et al., 2012, Ahn et al., 2015) (Figure 2.). Cytokines together with metalloproteinases act in the process of adhesion and implantation of endometrial fragments into the peritoneal surfaces (Sikora et al., 2012, Burney and Giudice 2012). RANTES (regulated on activation, normal T cell expressed and secreted) is a potent chemokine that attracts granulocytes and natural killer cells, and high concentrations of RANTES have been measured in the peritoneal fluid of endometriosis patients (Hornung et al., 1997). Inflammatory neurotrophins such as NGF seem to contribute to the increased nerve sprouting present in endometriosis. (Anaf et al., 2002, McKinnon et al., 2015).

Sampson's hypothesis of retrograde menstruation is supported by the observation that endometriosis is more common among patients who have vaginal obstruction of outflow (Olive and Henderson 1987). The more bleeding there is, the higher the risk of endometriosis; early menarche, and abundant, frequent menstruation seem to be risk factors for the development of the condition (Darrow et al., 1993).

Of the alternative pathogenetic theories, the coelomic-metaplasia theory proposes that endometriosis results from the differentiation of mesothelial cells into endometrium-like tissue (Vinatier et al., 2001). A third theory proposes that endometrial-like tissue fragments may migrate into the peritoneal cavity through lymphatic vessels and veins (Sampson 1927). This lymphatic spread could explain the occurrence of distant endometriotic foci that have been observed in the pericardium, pleura, and even in the brain (Noel et al., 2008). Interestingly, in a study by Noel et al. (2008), lymph node involvement was observed in 42% of patients with rectosigmoid endometriosis.

RVE has been suggested to have a pathophysiological origin that is different from other forms of endometriosis (Donnez et al., 1995). According to this theory, RVE originates from the Mullerian rests in the rectovaginal septum, and is actually "rectovaginal adenomyosis" (Donnez et al., 1995).

Figure 2. Mechanisms involved in the pathogenesis of endometriosis. EBAF=endometrial bleeding-associated factor; HGF=hepatocyte growth factor; VEGF= vascular endothelial growth factor; MMP=matrix metalloproteinase; TIMP= tissue inhibitor of metalloproteinase.



4.1. Central role of the endometrium

Almost every fertile-aged woman has some degree of retrograde menstruation, while only 6–10% of them develop endometriosis. Thus, it is intriguing to think that the explanation might be in the endometrium. Interestingly, the endometrium of a woman with endometriosis actually is abnormal in several ways. It shows numerous molecular and enzymatic alterations that finally result in increased synthesis of cytokines, prostaglandins, metalloproteinases and estrogen (for reviews see Giudice and Kao, 2004, Bulun, 2009) (Figure 2.) These alterations may take place even in normal endometrium as a result of oxidative stress in the pelvic cavity, and this may be one of the explanations why some women develop endometriosis while others do not (Yun et al., 2016). The changes in the endometrium are not only vital for implant survival, but they also help to create an inflammatory environment optimal for nerve sprouting (Stratton and Berkley 2011).

One of the fundamental features in endometriosis is the capacity of eutopic and ectopic endometrium to synthesize estrogen autonomously (Noble et al., 1997, Zeitoun et al., 1998). Aromatase is the enzyme that catalyzes conversion of androstenedione and testosterone, derived from ovarian and adrenal sources, to estrone, which in turn is converted to estradiol by 17 β -hydroxysteroid dehydrogenase 1. (Figure 3). This estrogen synthesis is increased in endometriosis patients while inactivation of estradiol to estrone by 17 β -hydroxysteroid dehydrogenase 2 is impaired (Huhtinen et al., 2012).

The overexpression of COX-2 (cyclooxygenase-2) in eutopic and ectopic endometrium results in increased prostaglandin E₂ (PGE₂) production, as COX-2 catalyzes the formation of PGE₂ from its precursor (arachidonic acid). This cascade is further stimulated by high concentrations of estradiol (Noble et al. 1997) (Figure 3). PGE₂ is involved in pain generation, causing uterine cramps and dysmenorrhea (Tsai et al., 2001, Sun et al., 2003).

A reduction of progesterone action in the endometrium, 'progesterone resistance' of women with endometriosis (Osteen et al., 2005) may also play a role in escalating estrogen actions. The effects of progesterone – such as downregulating estrogen receptors – are mediated through binding to progesterone receptor B; progesterone receptor A represses the function of receptor B. In endometriotic implants progesterone receptor B is lacking and only the inhibitory receptor A is present (Attia et al., 2000).

Figure 3.

Relationship between estrogen synthesis and inflammation (modified from Bulun, 2005, in Pharmacological Reviews).

A = androstenedione

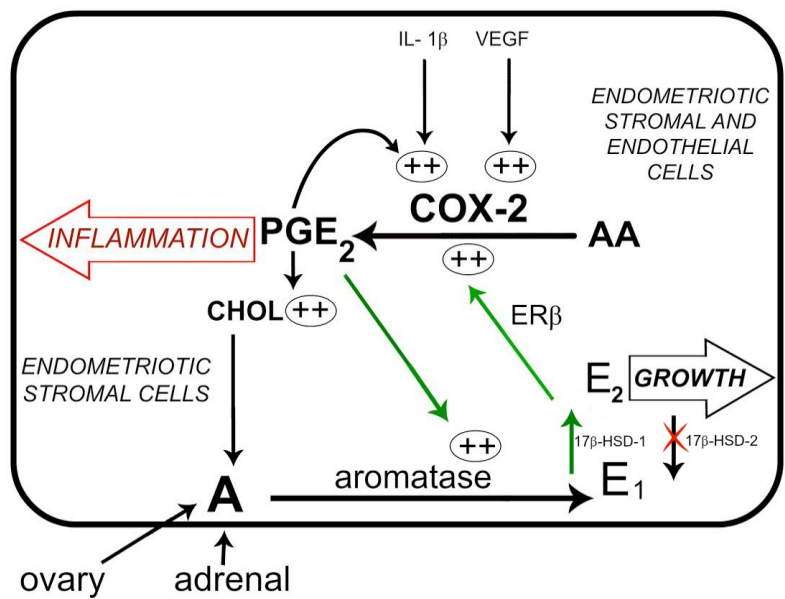
AA = arachidonic acid

E₁ = estrone

E₂ = estradiol

CHOL = cholesterol

ERβ = estrogen-receptor β



4.2. Nerve fibers in endometriosis

During the past few years, new evidence has emerged on innervation of endometriotic implants. Direct innervation of endometriotic implants by sensory and sympathetic nerve fibers was first identified in a rat model (Berkley et al., 2004) and later in lesions from women with endometriosis (Berkeley et al., 2005). In 2006, small unmyelinated nerve fibers were recognized in the endometria

of women suffering from endometriosis (Tokushige et al., 2006). These nerve fibers were lacking in the endometria of healthy controls. This finding of increased innervation was later also recognized in peritoneal lesions (Tokushige 2006). Furthermore, these nerve fibers were more frequently seen near the endometriotic glands and blood vessels than elsewhere in the stroma. By using immunohistochemical staining with specific markers (Table 2), it was demonstrated that these nerve fibers were a mixture of myelinated A δ , unmyelinated sensory C, cholinergic and adrenergic nerve fibers (Tokushige 2006). Recently, a predominance of sensory fibers (proinflammatory fibers) has been identified (Arnold et al. 2012).

Anaf et al. (2000) were the first to describe the presence of nerve fibers in women with rectovaginal endometriotic nodules. Women with more severe preoperative pain symptoms demonstrated a significantly higher density of nerve fibers within the endometriotic nodules. Thereafter, an increased density of nerve fibers in DIE lesions compared with peritoneal lesions was documented by Wang et al (2009). In particular, nerve fiber density was high in rectal lesions. Similar results of more nerve fibers in DIE compared with other sites were further confirmed by McKinnon et al (2012).

Table 2.

Typical markers used in immunohistochemical (IH) staining for identification of different nerve fibers.

Marker	Type of nerve fiber detected
Protein gene product 9.5 (PGP9.5)	pan-neuronal marker (all nerve fibers)
Neuropeptide Y (NPY)	adrenergic nerve fibers
Substance P (SP)	sensory nerve fibers
Vasoactive intestinal peptide (VIP)	cholinergic nerve fibers

4.3. Nerve fibers and pain – is there a connection?

The clinical relevance of finding nerve fibers in endometriotic lesions is still somewhat controversial. According to the results of a study by Maharajaa et al. (2014), a statistically significant positive correlation exists between nerve fiber density and perimenstrual pain scores.

In a pilot study by Meschner et al. (2009), patients with peritoneal endometriosis with more severe pain symptoms (visual analogue scale [VAS] score ≥ 3) appeared to have more endometriosis-associated nerve fibers in the peritoneum compared with patients with milder symptoms. This indicates that the excess of nerve fibers in endometriosis might be connected to clinical pain symptoms. Interestingly, in RVE nodules, there is a particularly rich innervation (Wang et al., 2009), and these women represent a group of patients with most distracting pain symptoms (Fauconnier and Chapron 2005). However, RVE often develops in anatomical sites that are richly innervated to begin with, such as the bowel wall or uterosacral ligaments. Therefore, hyperinnervation may only reflect a typical feature of these anatomical sites, and may not be connected to pain symptoms at all. McKinnon et al. (2012) have reported that the abundance of nerve fibers is associated with pain only in peritoneal endometriosis, and not in RVE.

Recent studies unanimously show that inflammation associated with endometriosis induces the release of neurotrophic factors (such as NGF), which then lead to the generation of nerve fibers, and possibly, pain (Maharajaa et al., 2014, McKinnon et al., 2015).

4.4. The origin of nerves – association between nerve and vascular growth

It is well accepted that angiogenesis, the development of new blood vessels from existing blood vessels, has an important role in the establishment and growth of endometriotic lesions (May and Becker 2008). VEGF and other angiogenic factors have been measured in high concentrations in peritoneal fluid from women with endometriosis (McLaren et al., 1996).

The growth patterns of both blood vessels and nerve fibers are very similar processes, which share common pathways (Carmeliet and Tessier-Lavigne 2005). VEGF and NGF are key mediators of both angiogenesis and neurogenesis (Becker et al., 2011). In addition, when new endometriotic lesions develop vasculature, the vessels bring along sensory and adrenergic nerve supplies.

Interestingly, different pathophysiological processes in endometriosis – neurogenesis, angiogenesis, inflammation – seem to be connected through the presence of NGF.

5. Peripheral and central pain mechanisms in endometriosis

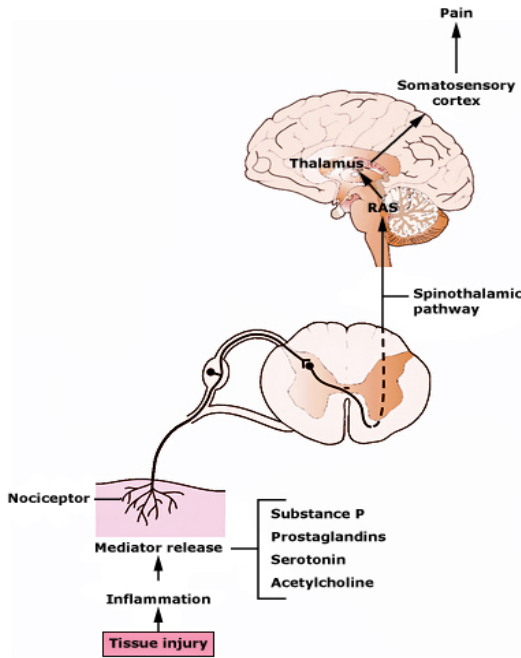
The local effects of ectopic endometriotic tissue such as cyclical bleeding or compression of nerves may contribute to pain generation in endometriosis (Anaf et al., 2000, Fauconnier and Chapron 2005). However, in recent years, the focus in endometriosis-associated pain research has moved from lesions towards understanding mechanisms involved in pain perception. The role of the CNS in this process is essential (Stratton and Berkley 2011). Transmission of pain stimuli to the CNS is illustrated in Figure 4.

The release of pro-inflammatory factors from endometriotic tissue and immune cells into peritoneal fluid activates the nociceptors in sensory C-nerve fibers (Stratton and Berkley 2011). During inflammation, sensory C-fibers may simultaneously convey information to the CNS (afferent function) and at the same time release inflammatory peptides into the local environment. Sometimes these sensory C-fibers may start discharging inflammatory agents through an efferent function without any noxious stimuli (Zhang et al., 2008). This peripheral sensitization is the main mechanism in the development of neuropathic pain present in endometriosis. Central sensitization occurs through neural mechanisms identical to those underlying memory, in which case central pain perception becomes autonomous of any peripheral input (Stratton and Berkley 2011, Morotti et al., 2014). Central sensitization may also explain why therapies targeted at the periphery, such as repeated surgery, may not relieve pain. Similarly, the return of pain after surgery is sometimes seen without evidence of new lesions (Abbott et al., 2003) hence reflecting the neuropathic nature of pain. Interestingly, estrogen may somehow modulate pain perception in the periphery and the CNS, and its effects appear to be both pro-nociceptive and anti-nociceptive (Craft 2007).

Pain is a stressful experience, and the hypothalamic-pituitary-adrenal (HPA) axis is also activated during pain. Stressful factors such as chronic pain result in increased secretion of CRH and vasopressin from the hypothalamus, which in turn stimulate pituitary ACTH secretion. This cascade leads to the release of glucocorticoids from the adrenals. Glucocorticoids have anti-inflammatory effects mediated mainly by suppression of cytokine activity (Joels et al., 2007).

Monoaminergic neurons in the brain stem usually inhibit nociceptive transmission. However, with HPA activation, an increase in glucocorticoid secretion induces monoamine depletion (for review see Blackburn-Munro G and Blackburn-Munro R, 2003) resulting in increased pain.

Figure 4. Nociception and pain transmission.



6. Non-hormonal medical treatment of pain

6.1 Anti-inflammatory analgesics

As the major symptom in endometriosis is pain, and the major pathophysiologic mechanism underlying this condition is inflammation, it is not surprising that nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely used for pain alleviation in endometriosis. The mechanism of action of NSAIDs involves inhibition of the enzymes COX-1 and COX-2, hence reducing the production of prostaglandins. However, to date, little evidence exists on the effects of NSAIDs on endometriotic pain (Allen et al., 2009). No studies have been conducted on the use of NSAIDs in RVE. Some serious side effects have been connected to NSAID use, including peptic ulcers, acute renal failure, and anovulation (Hayes and Rock 2002, Dunselman et al., 2014).

7. Hormonal treatment of pain

7.1 Use of GnRH analogues

The rationale of using GnRH analogues is based on the suppression of ovarian estrogen production through downregulation of GnRH receptors at pituitary level, thereby lowering gonadotropin secretion. As a result, profound hypoestrogenism with amenorrhea is achieved. Even though GnRH analogues appear effective in achieving pain relief, their efficacy is limited to the period of medication use, which usually is six months (Brown and Farquhar 2014). Moreover, side effects due to hypoestrogenism such as hot flushes, urogenital atrophy, loss of libido, deterioration of the lipid profile, and bone mass decrease occur commonly in connection with GnRH analogues (Prentice 2000). “Add-back therapy” with hormone replacement treatment may resolve these side effects and enable the use of GnRH analogues for longer periods of time. As GnRH analogues are also costly, progestogens and COCs have widely replaced them as the first-line treatment of endometriosis-associated pain.

7.2 Progestogens – why do they work?

The Practise Committee of the American Society for Reproductive Medicine (2008) has stated that “endometriosis should be viewed as a chronic disease that requires a life-long management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures”. Progestogens and COCs offer a good option for use on a long-term basis because of their good safety profile and low cost (Dunselman et al., 2014).

Progestogens have been used in the symptomatic treatment of endometriosis for decades. They can be used either alone or combined with estrogens, as in COCs. The most common side effects reported in connection with progestogens are weight-gain and menstrual irregularities.

Several interesting mechanisms of action have been recognized in connection with progestogens. They inhibit ovulation and thus suppress estrogen secretion from the ovaries in a dose-dependent manner. As endometriosis is estrogen-dependent, this mechanism offers a biological basis for their use. Progestogens also cause decidualization of the endometrium (Jones et al., 2000), and, therefore, antagonize estrogen actions. Subsequently, atrophy is achieved both in the eutopic endometrium

and in the ectopic implants. As endometriosis is thought to evolve as a consequence of retrograde menstruation and implantation of the regurgitated endometrium into the peritoneal surfaces (Sampson 1927), this mechanism of action may be fundamentally important in controlling the activity of disease and pain symptoms. Also, progestogens have anti-angiogenic (Blei et al. 1993) and anti-inflammatory properties (Zhao et al., 2002). The anti-inflammatory effect is most likely achieved by suppressing ovarian estrogen secretion, hence reducing estrogen-induced prostaglandin production (Bulun 2009).

The effect of COCs on regulation of apoptosis in the endometrium has been studied by Meresman et al., (2002). Eutopic endometrial cells from patients with endometriosis show an increased survival capacity as a result of high expression of the proto-oncogene *Bcl-2* (B cell lymphoma/ leukemia-2), which blocks apoptosis (Meresman et al., 2002). The protein Bax antagonizes this effect, and the ratio of Bcl-2 to Bax is important in determining susceptibility to cell death (Chao and Korsmeyer 1998). Administration of COCs appears to increase expression of the Bax gene, and consequently, apoptosis (Meresman et al., 2002).

Endometriotic lesions – and RVE in particular – are richly innervated by small nerve fibers (Tokushige et al., 2006, Wang et al., 2009). The use of progestogens and COCs reduces the density of these nerve fibers (Tokushige 2008, 2009), which may result from progestogen-mediated reduction of NGF expression (Mita et al., 2014). Therefore, this new mechanism of action may add to the favorable effects of progestogens, and together with their anti-inflammatory, anti-angiogenic and immunomodulatory properties, constitute the basis for the efficacy of progestogens and COCs in endometriosis.

7.3 Studies on progestogens and COCs in RVE

Even though progestogens and COCs are widely used for the medical treatment of endometriosis, studies on their use in cases of RVE are few (Table 3).

An intrauterine device that releases levonorgestrel at 20 µg/day over a 5-year period (the LNG-IUS) has a profound effect on the endometrium, rendering it atrophic and inactive (Nilsson et al., 1986). The endometrial decidualization has been described as being similar to that occurring during the first trimester of pregnancy (Chritchley et al., 1998). Therefore, a major reduction in menstrual bleedings occurs and amenorrhea is encountered in 20–30% of LNG-IUS users (Xiao et al., 1995). The LNG-IUS offers a safe, convenient, and inexpensive route for progestin-therapy, and it seems to offer

good symptom control in cases of peritoneal endometriosis (Vercellini et al., 2003, Lockhat et al., 2004, 2005), and RVE (Fedele et al., 2001). Moreover, the effect of the LNG-IUS on recurrent endometriosis-associated pain has been shown to be equally good as that associated with GnRH analogues (Petta et al., 2005). LNG-IUS use does not suppress ovulation and therefore levels of circulating estradiol remain within the range observed in women of reproductive age (Nilsson et al., 1980). The positive effects on pain therefore most likely result from local effects on ectopic endometrium, and possibly from reduction of retrograde menstruation.

Progestogens have also been compared with repeat surgery in the treatment of recurrent dyspareunia associated with DIE (Vercellini et al., 2012). Both treatment modalities were equally good and improved the quality of life, but the effects of progestogen became apparent more gradually over time, whereas surgery offered pain relief immediately and the effects diminished with time (Vercellini et al., 2012). New evidence shows that hormonal therapies reduce the size of rectovaginal nodules (Ferrero et al., 2013), which partly explains their efficacy in relieving pain.

Table 3. Studies (with > 20 patients) on progestogens and COCs in the treatment of RVE with or without bowel involvement.

EE2=ethinyl estradiol

NETA=norethisterone acetate

ETOG=etonogestrel

NG=norelgestromin

GES=gestodene

DEG=desogestrel

CPA=cyproterone acetate

DNG=dienogest

LNG-IUS=levonorgestrel-releasing intrauterine system

*Only patients with rectovaginal endometriosis considered

** All patients had colorectal endometriosis

Authors	Study design	No of patients	Intervention	Duration of treatment	Main result
Vercellini et al. 2005	Randomized controlled trial	90	EE2 10 µg+CPA 3 mg/day vs. NETA 2.5 mg/day	12 months	pain significantly reduced in both groups
Vercellini et al. 2010	Patient preference study	59*	Vaginal EE2 15µg+ETOG 120 µg/day vs. transdermal EE2 20µg+ NG 150µg/day	12 months	pain significantly reduced in both groups, ring more efficient
Ferrero et al. 2009	Patient preference study	82	letrozole 2.5 mg/day+NETA 2.5mg/day vs. NETA 2.5 mg/day	12 months	pain significantly reduced in both groups, combination more efficient
Ferrero et al. 2010	Prospective non-comparative	40**	NETA 2.5 mg/day	12 months	cyclical diarrhea and cramping reduced
Ferrari et al. 2012	Prospective non-comparative	26**	EE2 150µg+GES 60 µg/day	12 months	pain significantly reduced, lesions shrank
Morotti et al., 2014	Pilot open-label prospective study (after 6-months of unsuccessful NETA use)	25	DNG 2 mg/day	6 months	pain significantly reduced
Morotti et al., 2014	Patient preference study	144	DEG 75µg/day vs. COC	6 months	pain significantly reduced in both groups, DEG-group more satisfied
Maggiore et al. 2014	Patient preference study	143**	DEG 75µg/day vs. vaginal EE2 15µg+ETOG 120µg/day	12 months	symptoms significantly reduced in both groups, DEG more efficient

7.4 Aromatase inhibitors

As estradiol is excessively synthesized in endometriotic tissue through the action of aromatase, it is logical that aromatase inhibitors offer a good treatment option. Aromatase inhibitors suppress estrogen synthesis locally in endometrial and endometriotic tissue. Decreased estrogen levels stimulate the pituitary secretion of FSH and act as ovulation inducers. Hence, in fertile-aged women aromatase inhibitors need to be combined with progestins, COCs, or GnRH analogues (Ferrero et al., 2011). In RVE, letrozole, 2.5 mg daily combined with norethisterone acetate (2.5 mg/day) has proved beneficial (Remorgida et al., 2007, Ferrero et al., 2009). However, such combination therapies among fertile-aged women are not the first-line therapies, as beneficial effects can also be achieved by using monotherapy with progestogens or COCs.

8. Other medical options in endometriosis

During the past few decades, several promising pharmacological agents – such as immunomodulators, selective progesterone receptor modulators (SPRMs), GnRH antagonists, antiangiogenic agents, and melatonin – have been tested regarding their efficiency in relieving endometriosis-associated pain.

The use of GnRH antagonists in the treatment of endometriosis pain has recently been introduced. GnRH antagonists have a molecular structure that enables their use orally (Ezzati and Carr, 2015). They reduce estrogen levels without triggering severe side effects, as the initial peak in gonadotropin secretion is lacking (Kupker et al., 2002).

SPRMs such as mifepristone, ulipristal acetate and asoprisnil represent a group of progesterone receptor ligands that display progesterone agonist, antagonist, or mixed agonist–antagonist effects depending on the target tissue. Their daily use induces amenorrhea by mechanisms that are partly

unknown. It seems that they inhibit ovulation without reducing the levels of circulating estradiol (Chabbert-Buffet et al., 2012). Early studies have shown promising results as regards their efficacy and tolerability in managing endometriosis-associated pain (Chwalisz et al., 2005).

The antiangiogenic agent bevacizumab (Avastin) has proved beneficial in reducing the growth of endometriotic implants in mice, and, therefore, may offer a novel therapeutic option sometime in the future (Ricci et al., 2011). Overall, a huge amount of pharmacological agents have been tested for the treatment of endometriosis, but none of these in cases of RVE (Vercellini et al., 2008, Zito et al., 2014, Ferrero et al., 2015). Moreover, medical therapy as a treatment option for RVE has aroused interest only during the past few years.

9. Surgical treatment of RVE

9.1 Radical resection is the main goal

In patients who do not respond to hormonal therapies, or among patients with side effects from hormone use or those who wish for pregnancy, the gold standard for the treatment of symptomatic RVE is surgery (Abbott et al., 2004, Chapron et al., 2004, Dunselman et al., 2014). RVE is not considered a progressive disease and therefore asymptomatic patients do not need to undergo operation (Koninckx et al., 2012). Surgery is in theory based on straightforward oncologic principles, i.e. radical excision of all visible and palpable lesions (Abbott et al., 2004, Chapron et al., 2004, deCicco et al., 2011, Koninckx et al., 2012). The posterior vaginal wall may need to be resected if endometriosis infiltrates the posterior fornix. The surgical procedure is described in detail in Publication I. As most patients affected by endometriosis are of fertile age, it adds to the challenges of RVE surgery – the surgery needs to be radical but at the same time as conservative as possible, as future reproductive performance should not be negatively influenced.

9.2 Diagnosis and preoperative assessment of RVE

Rectovaginal endometriosis should always be suspected among women of fertile age with invalidating dysmenorrhea, deep dyspareunia or typical bowel symptoms. By way of clinical examination, approximately 50% of DIE lesions over 3 cm in diameter can be diagnosed (Koninckx et al., 1996). Laparoscopy is the gold standard for the diagnosis of endometriosis. However, as RVE noduli are located retroperitoneally, and often associated with cul-de-sac obliteration, magnetic resonance imaging (MRI) should be used preoperatively as a diagnostic tool to identify all RVE noduli and possible colorectal involvement. Colorectal involvement can also be diagnosed with the use of ultrasonography (US). The sensitivity and specificity of US in diagnosing colorectal involvement have been reported to be as high as 85–100% (Hudelist et al., 2011), but in real life it seems that the accuracy of US varies according to the expertise of the user. MRI has proved to be accurate in the diagnosis of RVE and bowel involvement (and the degree of bowel stenosis) (Bazot et al., 2004). MRI can also be a useful tool in the assessment of possible ureteral involvement and hydronephrosis.

9.3 rAFS classification of endometriosis is used in surgery

During surgery, the severity and extent of endometriosis should be classified. The most commonly used classification system was proposed in 1979 by the American Fertility Society. A few years later, the classification system was revised (rAFS 1985). This revised American Fertility Society (rAFS) classification divides endometriosis into four categories (I–IV) depending on the severity of the disease (where rAFS I is mild endometriosis and rAFS IV is severe endometriosis). This classification system was originally meant to predict future fertility – the highest scores result from adnexal involvement of endometriosis whereas deep lesions do not necessarily result in advanced staging III–IV (unless the cul-de-sac is obliterated). Therefore, several attempts have been made to develop a better classification system that would systematically take into account the DIE lesions (Adamson 2011).

The ENZIAN classification system was developed for better description of DIE and its involvement in the pelvic structures and organs, and it supplements the rAFS score (Haas et al., 2011). However, use

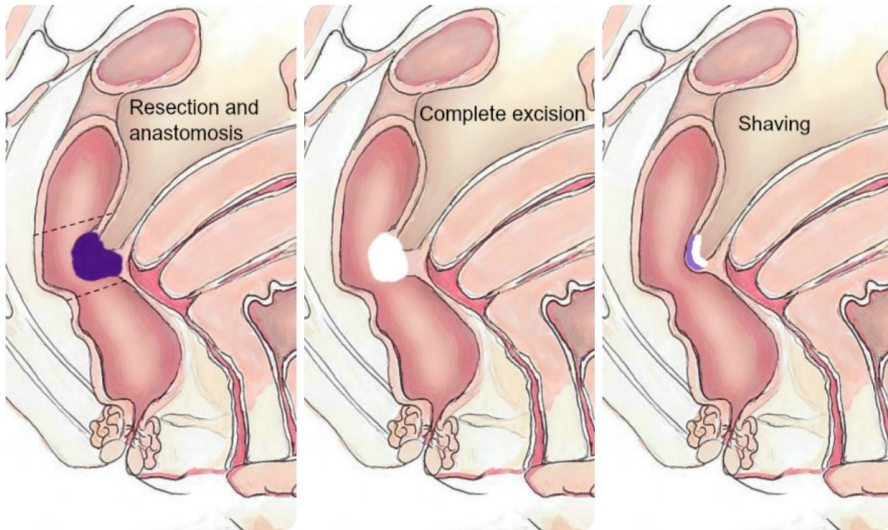
of the ENZIAN classification is somewhat complex, and therefore it has not achieved great popularity, and for the majority of cases rAFS scoring is still used.

9.4 RVE with colorectal involvement – surgical options

RVE extending to the bowel wall is estimated to be present in 4–12% of RVE cases (Jerby et al., 1999, Remorgida et al., 2007, Wills et al., 2008). The rectosigmoid colon is the most common site of gastrointestinal involvement (Zanetti-Dällenbach et al., 2008). Even though surgical management is considered the primary treatment for symptomatic RVE with colorectal extension (Garry 2004, Emmanuel and Davis, 2005), the most appropriate approach still remains controversial. Laparoscopy has evolved tremendously over the past decades and a laparoscopic approach to colorectal surgery in RVE has become the method of choice during the past few years (Jerby et al., 1999, Darai et al., 2010, Meuleman et al., 2011, Ruffo et al., 2012, Ruffo and Rossini 2013, Wolthuis et al., 2014).

Different surgical options include shaving (excision without opening the bowel wall), full-thickness disc excision (defined as excision with opening of the bowel wall followed by its closure) or bowel resection (and anastomosis) (Figure 5). The choice of the operative technique depends not only on the extent and depth of bowel endometriosis, but also on the surgical choice of the center. Some groups seem to favor bowel resection as the treatment of choice (Duepre et al., 2002, Darai et al., 2005, Dubernard et al., 2006) whereas others avoid bowel resection whenever possible (Donnez et al., 1997, Vercellini et al., 2006, Koninckx et al., 2012). It seems that in cases with large (over 3 cm) or multiple nodules, discoid resection often results in incomplete excision of endometriotic lesions, and therefore, symptom relief may not be achieved (Remorgida et al., 2005). In addition, excision of such large noduli may result in stenosis of the bowel lumen (Abrao et al., 2015). On the other hand, in less extensive cases, discoid resection has been reported to be a safe alternative to bowel resection, with less postoperative morbidity (Fanfani et al., 2010). More conservative surgical techniques (shaving, excision) have been suggested to lead to better functional outcomes in women with rectal endometriosis (Roman et al., 2013). Currently, however, 70% of patients who need colorectal surgery for RVE undergo bowel resection, and in the majority of cases, via laparoscopy (for review see Meuleman et al., 2011).

Figure 5. Surgical options for removing RVE noduli.



10. Complications of RVE surgery

The rates of major intra- and postoperative complications following laparoscopic surgery for DIE in large specialized centers vary from 2.1 to 13% (Vercellini et al., 2009, Kondo et al., 2010, Milone et al., 2015). Colorectal surgery increases the risk of complications; the overall complication rate following bowel resection in cases of RVE (either in laparoscopy or in laparotomy) is 22% – major complications occur in 11% of women (for review see DeCicco et al., 2010). Therefore, colorectal resections should always be performed by colorectal surgeons. Risk factors for the development of major complications in bowel resections are opening of the vagina at the time of surgery, and the treatment of low rectal lesions (< 5–8 cm from the anal verge) (Ruffo et al., 2010, Meuleman et al., 2011). In these cases, protective loop ileostomy may be needed (Wolthuis et al., 2014).

The majority of severe complications in colorectal surgery are bowel complications such as inadvertent rectal perforation or anastomotic leakage (Oliveira et al., 2015). The second most frequent major complication is rectovaginal fistula formation, with a 0–10% risk (Duepre et al., 2002, Darai et al., 2005). Other major complications include intra-abdominal bleeding, urinary tract injuries or fistulas, and pelvic abscesses and collections. Colostomy is often needed to treat severe

bowel complications, and the decision to possibly perform a bowel resection should be discussed and agreed upon with a patient prior to the operation.

The most common minor postoperative difficulty is urinary retention, probably due to damage to the parasympathetic plexus, resulting in temporary bladder denervation. This problem often occurs in connection with colorectal surgery (Dubernard et al., 2008). Nerve-sparing techniques have been suggested in order to avoid bladder dysfunction (Volpi et al., 2004, Possover et al., 2005, Landi et al., 2006). In these techniques, the key point is to dissect the posterior parametrium accurately so that damage to the hypogastric inferior nerve plexus is avoided. This can be done by starting the dissection from the side with more extensive disease, therefore leaving the nerves on the “better” side untouched.

11. Effect of surgery on pain and quality of life

Since 2000, there have been numerous studies carried out to evaluate the effect of RVE surgery on pelvic pain symptoms (for review see Vercellini et al., 2009). However, the definition and extent of RVE reported in the literature have been highly variable (RVE or RVE with colorectal involvement) and the same applies to the surgical procedure (laparotomy, laparoscopy, bowel surgery performed or not). Vercellini et al. (2006) compared surgery with expectant management in RVE patients suffering from pain symptoms and infertility. A statistically significant delay in symptom recurrence was observed in favor of the surgery group. The benefit of surgery was particularly evident as regards deep dyspareunia and dyschezia.

Overall, surgery for RVE seems to result in long-term pain relief (Fedele et al., 2004) and improved quality of life (Garry et al., 2000, Abbott et al., 2004, Ford et al., 2004, Setälä et al., 2012). Among women with RVE extending into the colorectum, pain relief following laparoscopic colorectal surgery is achieved in up to 85% of women in 12 months' follow-up time (Bassi et al., 2011). Laparoscopic colorectal resections also result in significantly improved quality of life (Table 4).

Recurrence rates following RVE surgery, defined as recurrence of lesions, or recurrence of pain, vary between 5–30% (DeCicco et al., 2011, Meuleman et al., 2011, Koninckx et al., 2012). Bowel resection seems to reduce the risk of symptom recurrence (Fedele et al., 2004), probably due to the completeness of resection. In women with recurrent disease, pain alleviation is usually achieved with hormonal therapy (Vercellini et al., 2003). If repeat surgery is considered, hysterectomy with bilateral oophorectomy produces the best results (Vercellini et al., 2009).

Table 4. Studies carried out to assess the quality of life following laparoscopic colorectal resection.

Study	Design	No. of patients	QoL measured by	Follow-up time (months)	Result
Dubernard et al., 2006	Prospective	58	SF-36	22.5	QoL↑ Tenesmus, constipation not improved
Bassi et al., 2011	Prospective	151	SF-36	12	QoL↑
Meuleman et al., 2011	Retrospective	45	Oxford Endometriosis QoL Questionnaire	27	QoL↑
Kössi et al., 2013	Prospective	26	15-D	12	QoL↑ , sexual well-being improved
Ribeiro et al., 2014	Prospective	40	SF-36	12	QoL↑
Silveira da Cunha Araujo et al., 2014	Prospective	45	SF-36	48	QoL↑
Touboul et al., 2015	Prospective RCT	26 *	SF-36	51	QoL↑ in both groups
Kent et al., 2015	Prospective	137	EQ-5D	12	QoL↑, pelvic clearance** improved outcome

SF-36=Short form-36 Health status and the quality of life survey

EQ-5D=Euro Quality of Life Five Dimensions Questionnaire

* Bowel resections ($n=52$) undertaken via laparoscopy ($n=26$) or laparotomy ($n=26$) were compared

**Pelvic clearance=hysterectomy with bilateral salpingo-oophorectomy

12. Role of postoperative hormone treatment in RVE

Regarding RVE, no studies have been conducted on the postoperative use of HT in the prevention of symptom recurrence. According to the results of a pilot study by Vercellini et al. (2003), postoperative insertion of an LNG-IUS significantly decreased the risk of symptom recurrence compared with endometriosis surgery only. However, the patients were not suffering from DIE. Recently, Bayoglu Tekin et al. (2011) compared the efficacy of a postoperatively inserted LNG-IUS to GnRH analogue treatment (for six months) in cases of severe endometriosis (rAFS stage IV). Both modalities showed comparable effectiveness in reducing pain during the 12-month follow-up time (Bayoglu Tekin et al., 2011). Moreover, postoperative COC use has been shown to decrease the risk of ovarian endometrioma recurrence (Vercellini et al., 2013). For these reasons, it has been suggested that the LNG-IUS and combined oral contraceptives may be helpful in preventing the recurrence of dysmenorrhea after surgery (Abou-Setta et al., 2006, Somigliana et al., 2014). New ESHRE guidelines on the treatment of endometriosis favor their use postoperatively (Dunselman et al., 2014).

AIMS OF THE STUDY

This study was undertaken to investigate the long-term effects of surgery in the treatment of RVE. In addition, the significance of nerve fibers in RVE-associated pain was investigated.

Specific aims of the study were:

1. To study the long-term outcome of surgery in terms of pain recurrence
2. To identify factors associated with recurrence
3. To identify factors predisposing to bowel resection
4. To study the feasibility of laparoscopic access as regards bowel resection
5. To study the presence of nerve fibers in cases of RVE, and the effect of hormonal therapies on their density

MATERIALS AND METHODS

Approval to perform this study was granted by the Hospital District of Helsinki and Uusimaa (6.4.2004 Dnro 125/E9/06).

1. Surgical data on RVE (Studies I, III)

Study I covered all operations performed for RVE – with or without bowel resection – between January 2000 and May 2004 at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital ($n = 153$). Study III was focused on bowel resections; therefore, only operations in which bowel resection was performed were included in the time period from June 2004 until the end of 2012 ($n = 164$).

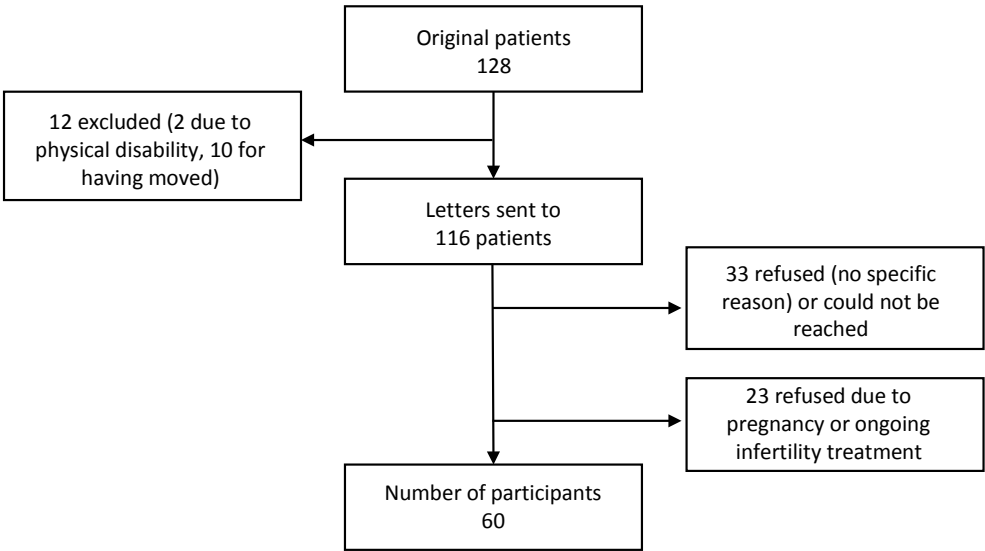
The patient data were obtained from hospital charts. The demographic data included age and BMI at the time of surgery as well as history of pregnancies, contraception, other hormonal therapies, symptoms, and prior operations.

Surgical data included the method of access (laparoscopy or laparotomy), operative details, rAFS (revised American Fertility Society) classification (1985), and peri-operative and postoperative complications. Regarding operations with bowel resection (Study III), data on the length of resected bowel, the size and number of noduli in resected bowel, the level of anastomosis and the need for ileostomy or colostomy were obtained. Data concerning fertility and reoperations following surgery were also collected. In addition, the rate of complications (“learning curve”) associated with the main gynecologic surgeon in performing laparoscopic bowel resections was studied.

2. Patients in Study II

In order to evaluate the long-term effects of surgery, and especially the factors associated with recurrence, patients operated upon between January 2002 and May 2004 were offered a follow-up visit at the clinic, as shown in Figure 6.

Figure 6. Patients in Study II.



Prior to assessment at the clinic, patients were asked to fill in a symptom diary for 30 consecutive days. The severity of dysmenorrhea, deep dyspareunia, non-menstrual pelvic pain, and dyschezia were separately evaluated each day using a visual analogue scale (VAS). The amount of uterine bleeding was also assessed each day using a 4-point scale (0 = no bleeding, 1 = spotting, 2 = light bleeding, 3 = regular bleeding, and 4 = heavy bleeding).

At the follow-up visit, recurrence of the disease was diagnosed clinically on the basis of pelvic examination. Data on hormonal therapies used, pregnancies and reoperations were collected.

Current symptoms were evaluated on the basis of symptom diaries. A sum score for each symptom and each patient was calculated from 30 daily VAS assessments (0–10) by adding the daily VAS scores. Thus, the maximum sum score for each symptom was 300. In addition, an overall symptom sum score was obtained as a mean of the four separate symptom scores. Similarly, a bleeding sum score was obtained from the daily bleeding assessments (0–4), resulting in a theoretical maximum of 120.

3. Tissue samples and immunohistochemistry (Study IV)

In order to study the types and densities of nerve fibers in RVE, permission to reexamine samples removed during the index surgery was obtained at the time of the follow-up visit. Altogether, 45 RVE specimens were eligible for reexamination, and subjected to immunohistochemical staining, as shown in Table 5. The types and densities of nerve fibers were thereafter analyzed as regards the association of nerve fibers with various patient characteristics such as hormone use.

Table 5. List of antibodies and optimal dilutions used in immunohistochemical staining.

Antibody*	Antigen target retrieval	Dilution of primary antibody	Marker of
PGP9.5	pH 9	1:1,400	all nerve fibers
NPY	pH 6	1:8,000	sympathetic fibers
VIP	No Target Retrieval	1:5,000	parasympathetic fibers
SP	pH 9	1:8,000	sensory fibers
NGF	pH 9	1:500	
TrkA	pH 9	1:500	main receptor of NGF

*All antibodies were supplied by Dako, Glostrup, Denmark

4. Statistical analysis

Univariate and multivariate association models were used to identify the risk factors for bowel resection (Study I).

The factors associated with clinical recurrence (Study II) were evaluated by using univariate and multivariate logistic regression models. Odds ratios (ORs) with 95% confidence intervals (CIs) and *p*-values were reported. The statistical analyses were performed by StatFinn Oy (Espoo, Finland). The statistical software used was SAS V 9.1 for Windows.

In Study III, statistical analysis was performed using PASW 18.0 for Mac (SPSS Inc., Chicago, IL, USA). In Study IV, the data were analyzed using IBM SPSS 19.0 Statistics (Armonk, New York, USA). Differences in continuous variables were analyzed by means of the Mann–Whitney *U*-test for skewed data. The chi-squared test was used for independent nominal data, presented as *n* (%). Three levels of anastomosis (low, medium or high) as well as three 3-year periods (2004–2006, 2007–2009 or 2010–2012) were compared by using the chi-squared linear-by-linear association test. The level of statistical significance was set at $p < 0.05$.

RESULTS

1. General aspects

Between January 2000 and May 2004, a total of 153 operations were performed for RVE; 57 patients (37%) were treated laparoscopically, and 96 patients (63%) via laparotomy. Fifty-four patients (35%) underwent bowel resection. There were four major complications (2.6%) requiring further surgery. Patient characteristics are shown in Table 6. All patients were symptomatic; the leading symptom was dysmenorrhea.

Table 6. Characteristics of 153 patients operated upon because of RVE in 2000–2004.

	<i>n</i> =153	
Age (years) (median (range))	31	(20–49)
<i>Symptoms:</i>		
Dysmenorrhea	92	(60%)
Pelvic pain	56	(37%)
Dyspareunia	55	(36%)
Dyschezia or rectal bleeding	68	(44%)
Infertility	45	(29%)
Previous surgery for endometriosis	84	(55%)
Previous surgery for RVE	18	(12%)
HT use preoperatively*	78	(51%)
rAFS stage	I	2 (1%)
	II	56 (37%)
	III	34 (22%)
	IV	61 (40%)

*HT=hormonal therapies (progestogens or combined oral contraceptives)

2. Risk factors of bowel resection

The risk of bowel resection was increased among patients with previous surgery for endometriosis, intestinal symptoms, or rAFS score IV. Preoperative use of combined oral contraceptive pills was associated with a lower risk of bowel resection (Table 7).

Table 7.

Univariate associations between various demographic, preoperative and perioperative factors, and bowel resection. The data are presented as odds ratios (ORs) with 95% confidence intervals (CIs).

Factor	OR	Lower 95% CI	Upper 95% CI	p-value
Age	1.02	0.96	1.08	0.47
History of infertility	1.30	0.63	2.66	0.48
Previous surgery	2.74	1.35	5.54	0.005
Preoperative use of COCs (vs. no contraception)	0.32	0.15	0.66	0.002
Preoperative symptom				
Dysmenorrhea	0.84	0.43	1.65	0.61
Pelvic pain	0.91	0.46	1.82	0.79
Dyspareunia	0.57	0.28	1.16	0.12
Dyschezia or rectal bleeding	2.55	1.29	5.02	0.007
rAFS III (vs. I+II)	2.04	0.77	5.41	0.15
rAFS IV (vs. I+II)	4.71	2.06	10.78	0.0002

3. Recurrence following RVE surgery

Altogether, 60 patients participated in the follow-up study. The mean time (\pm SD) from index surgery to clinical evaluation was 4.0 ± 0.5 years. Patients presented with low symptom sum scores on the basis of symptom diaries ($n=51$). The median symptom sum scores (max 300) for each symptom are presented in Table 8.

Table 8. Median symptom sum scores (range) in 51 symptom diaries for each endometriosis-associated symptom. The sum score for each symptom was calculated by adding 30 daily VAS scores (0–10) (theoretical maximum of 300).

Symptom	Median symptom sum score (range)
Dysmenorrhea	3 (0-32)
Pelvic pain	9 (0-72)
Dyspareunia	0 (0-52)
Dyschezia	2 (0-76)

Evidence of RVE recurrence on pelvic examination was found or suspected in 29 (48%) of the subjects. Detection of clinical recurrence was not associated with pain symptoms. In univariate analysis, amenorrhea (bleeding sum score = 0 in the symptom diary) at the time of clinical assessment was associated with a lower risk of recurrence. Undergoing bowel resection was of borderline significance in protecting women from recurrence. In multivariate analysis, the protective effect of bowel resection was significant (Table 9).

Table 9. Factors associated with clinical RVE recurrence.

Each symptom was considered prevalent if the symptom sum score exceeded 3. The symptom sum score was calculated by adding 30 daily VAS scores (0–10) (theoretical maximum of 300).

HT = hormonal therapies

Factor	Univariate analysis	Multivariate analysis
	OR (95% CI), p-value	OR (95% CI), p-value
Age	0.92 (0.82–1.03), 0.14	
Smoking	1.05 (0.33–3.30), 0.94	
Previous endometriosis surgery	0.59 (0.21–1.66), 0.32	
Bowel resection at index surgery	0.37 (0.13–1.07), 0.07	0.23 (0.06–0.89), 0.03
HT postoperatively	2.31 (0.81–6.54), 0.12	
Prevalent dysmenorrhea	1.31 (0.38–4.50), 0.67	
Prevalent pelvic pain	0.71 (0.23–2.22), 0.56	
Prevalent dyschezia	0.59 (0.16–2.09), 0.41	
Amenorrhea	0.13 (0.02–0.65), 0.01	0.17 (0.02–1.56), 0.12

4. Outcome of colorectal surgery

Between June 2004 and December 2012 a total of 164 rectosigmoid resections for RVE were performed, of which 112 (68%) were undertaken laparoscopically and 52 (32%) by way of laparotomy. The proportion of laparoscopic bowel resections increased towards the end of the study period. The rate of major complications was 12% both in laparoscopies and in laparotomies. The rate of complications in laparoscopies decreased from 27% in 2004–2006 to 8% between 2010 and 2012 (Table 10). Similarly, the complication rate associated with the main gynecological surgeon in laparoscopic bowel resections decreased with increased personal experience.

A greater size (≥ 4 cm) of a nodule in resected bowel was significantly associated with the development of a major complication (Table 10). During the median follow-up period of 61 months (range 16–116 months) 7% of cases needed reoperation due to recurrence. Forty-seven percent of those women who preoperatively desired a pregnancy subsequently had a child.

Table 10. Factors associated with major complications in 164 colorectal resections. (The level of anastomosis was that measured from the anus.)

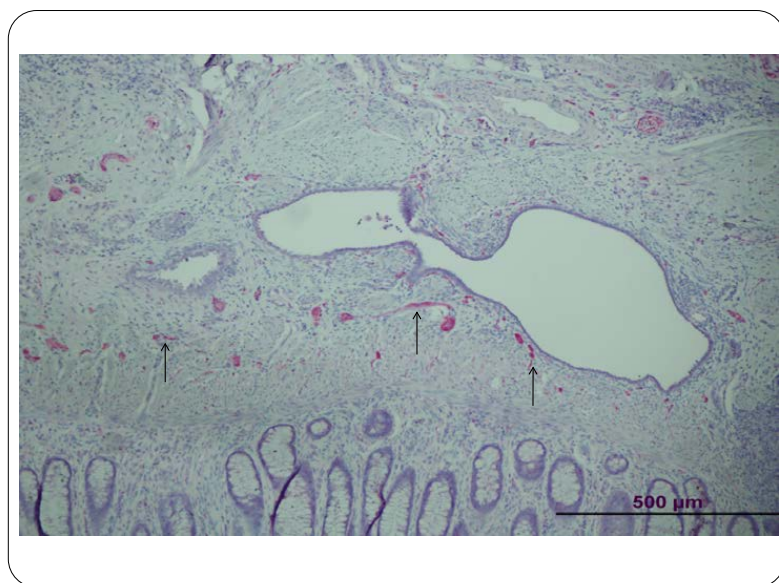
Variable	n (%)	Number of complications (%)	p-value
Nodule size < 4 cm	87 (53%)	6 (7%)	0.04
Nodule size ≥ 4 cm	68 (41%)	12 (18%)	
Low anastomosis (≤ 5 cm)	15 (9%)	3 (20%)	0.26*
Medium anastomosis (6–9 cm)	102 (62%)	12 (12%)	
High anastomosis (≥ 10 cm)	47 (29%)	4 (9%)	
Operation during 2004–2006	49 (30%)	10 (20%)	0.04*
Operation during 2007–2009	48	4 (8%)	
Operation during 2010–2012	67	5 (7%)	
Laparoscopy	112	13 (12%)	
Laparotomy	52	6 (12%)	

*Chi-squared linear-by-linear association

5. Nerve fibers and the effects of HT

Altogether, 45 RVE specimens removed in surgery were immunohistochemically analyzed regarding the presence and density of nerve fibers. The use of hormonal therapy was associated with reduced densities of sympathetic, parasympathetic and sensory nerve fibers in DIE lesions. The density of total nerve fibers (assessed by using pan-neuronal marker PGP9.5; see Figure 7) was significantly lower ($p < 0.05$) in lesions collected from women receiving HT ($8.6/\text{mm}^2$, $4.2\text{--}20.8/\text{mm}^2$; median density, from 25th to 75th quartile) compared with that in lesions from untreated women ($24.9/\text{mm}^2$, $11.2\text{--}34.9/\text{mm}^2$). This is illustrated in Figure 2 in Publication IV. RVE lesions stained strongly for NGF and its receptor Trk-A. Expression of NGF, but not that of Trk-A, was significantly reduced during use of hormonal therapy.

Figure 7. Immunohistochemical staining of endometriotic tissue with the pan-neuronal marker PGP9.5 (dilution 1 : 1400) using Fast Red chromogen to identify nerve fibers (see arrows).



DISCUSSION

Deep infiltrating RVE is one of the most challenging conditions in the field of gynecology. It causes a large amount of suffering and pain, and affects women in their best reproductive years. The mechanisms of pain generation are poorly understood. Surgery is demanding, with a risk of severe complications. The significance of bowel resection is controversial. We conducted this study to clarify these compelling aspects with an emphasis on evaluating the long-term effects of surgery in patients with RVE. Moreover, the effects and mechanisms of HT in RVE were to be studied, as only a few studies exist on the use of HT in RVE-associated pain.

The ultimate origin of endometriosis-associated pain may remain a mystery, but nevertheless, several mechanisms for the development of pain have recently been recognized. It seems that endometriosis is a complex neuroinflammatory disease, and inflammatory mediators such as NGF seem to have a central role in generating pain (Stratton and Berkley 2011). NGF mediates inflammation, and induces the growth of vessels and nerve fibers in the endometriotic tissue (McKinnon et al., 2015). Moreover, NGF seems to have a key role in sensitizing nerve endings (Anaf et al., 2000).

Even though most hormonal therapies reduce inflammation, our study shows that the expression of NGF and its receptor Trk-A are only partially suppressed during the use of HT – a fact that could possibly explain the limited efficacy of HT in some endometriosis patients. Interestingly, as estrogen is known to stimulate NGF secretion, the autonomous estrogen synthesis in endometriotic tissue may be the fundamental mechanism in maintaining inflammation and persistent pain in patients not responding to HT.

New evidence shows that HT may, however, work better than has been previously thought in RVE-associated pain (Vercellini et al., 2009, Maggiore et al., 2014). Most forms of HT (COCs and progestogens) suppress ovulation and therefore reduce the levels of circulating endogenous estradiol. Endometriosis being estrogen-dependent, this mechanism of action seems fundamentally important. In addition, systemic progestogens also seem to have local effects as they reduce the size of RVE nodules (Vercellini et al., 2003, Ferrero et al., 2013). This local effect may be the result of decidualization and the subsequent atrophy of endometriotic tissue (Jones et al., 2000).

Interestingly, the LNG-IUS has also proved to work well in recurrent endometriosis pain (Petta et al., 2005). Therefore, ovulation inhibition may not be the central mechanism of action of all hormonal therapies – the majority of LNG-IUS users experience ovulations by the end of the first year of use (Nilsson et al., 1984). Reduction of retrograde menstruation – and the subsequent reduction of shed endometrium in the abdominal cavity – may play a crucial role in achieving pain-relief (Petta et al., 2005). Eutopic endometrium in endometriosis patients has several proinflammatory properties that are absent in healthy endometrium (Bulun, 2009).

Inflammatory processes also contribute to nerve sprouting, present to a great degree in endometriosis (Stratton and Berkley, 2011). Increased densities of sympathetic, parasympathetic and sensory nerve fibers have been identified in endometriotic lesions, and the innervation seems to be exceptionally dense in cases of RVE (Morotti et al., 2014). Our studies further confirm these findings. Interestingly, nerve fiber densities in RVE samples (removed at surgery) from hormone-users (progestogens, COCs) were lower than those in samples from non-users. This same finding of HT reducing the density of nerve fibers has been shown in previous studies (Tokushige et al., 2008, 2009). Quite possibly, this may be one of several mechanisms of action of HT in reducing pain symptoms in endometriosis. The partial suppression of NGF may be one of the explanations for the reduction in the densities of nerve fibers.

Surgery is needed when conservative treatment with HT does not lead to pain alleviation. Our results show that surgery for RVE is beneficial as regards long-term pain relief. The majority of patients who have undergone an operation for RVE remain symptom-free in the following years. Their reproductive performance also seems reasonably good, as almost half of those who wish for pregnancy succeed in their attempts. Interestingly, the majority of patients with persisting endometriosis symptoms after the operation have no clinical findings of RVE. This same finding has been documented in an earlier study (Abbott et al., 2003). This may imply that persistent pain in some cases is neuropathic in nature.

According to our results, in one third of RVE operations colorectal surgery is performed. This is in line with the results of previous studies (Jerby et al., 1999, Remorgida et al., 2007). During the past ten years the approach as regards colorectal surgery in RVE patients has changed. Most operations for RVE – with or without bowel involvement – are currently approached via laparoscopy. This shift towards a laparoscopic approach was also seen in our institute during our study period of 2000 to 2012. Laparoscopy offers fast and less painful recovery for patients, and it offers an optimal and precise view of the pelvic anatomy for the operating surgeon. However, laparoscopy for RVE demands expertise and repetition. In our study, we showed that the rate of complications is

associated with the experience of the operating surgeon. In addition, it seems that the rate of complications is higher in bowel resections performed because of RVE compared with bowel resections performed for other reasons (Dalton et al., 2009). Therefore, as RVE is not very common, centralizing these operations is essential in order to achieve the best possible results. Moreover, all laparoscopic bowel resections should be performed by colorectal surgeons in order to minimize the rate of complications. This has always been the practice in our institution.

Complication rates in the operations involving bowel resection in our study were not as high as those reported previously; rates as high as 22% have been reported (deCicco et al., 2011). We found that major complications develop in 12% of these operations performed either via laparoscopy or laparotomy – the risk of complications increases with more extensive disease (large or multiple nodules). However, these complications are potentially life-threatening, and in most cases demand colostomy for treatment in otherwise healthy young patients suffering – after all – from a benign condition. Therefore, it is understandable that some groups advocate a more conservative approach to bowel resection and favor performing shaving or, in cases of lesions penetrating the bowel wall through the muscularis layer, full-thickness discoid excision instead of bowel resection (Koninckx et al. 2012). It has been suggested that a more conservative approach to bowel surgery might result in better functional outcomes, and therefore, shaving and discoid excision should probably be considered in RVE with a nodule not larger than three centimeters (Remorgida et al., 2005, Roman et al., 2013).

Even though the most appropriate approach to bowel surgery may remain controversial, the fact is that in two thirds of patients who need colorectal surgery the choice currently is rectosigmoid resection (Meuleman et al., 2011). Our study proved that bowel resection is very likely to take place if the preoperative symptoms include rectal bleeding or pain during defecation. In addition, patients with a history of previous operations for RVE are at increased risk of needing colorectal surgery. This undoubtedly implies that prior surgery has not been radical enough for adequate symptom relief.

As recurrence – meaning recurrent pain or lesions – following surgery represents one of the major concerns in the surgical treatment of RVE, it is important to take all possible measures in order to reduce their number. Recurrence of DIE can be considered to be a result of incomplete surgery (Koninckx et al., 2012). Even though performing bowel resection carries risks in terms of complications, we found that it seems to have a protective effect against recurrences, most likely because of the completeness of resection. This finding is in accordance with that in an earlier study (Fedele et al, 2004). During the past ten years, endometriosis surgery has evolved greatly. Instead of patients undergoing several minor operations, the current aim is to carry out one major operation by

a multidisciplinary team – and to be as radical as possible without compromising future fertility. This approach has already been shown to reduce the rates of recurrence (Carmona et al., 2009).

All treatments – either surgical or hormonal – resulting in the absence of menstrual bleeding seem to protect women from recurrences. Therefore, hysterectomy should be considered for women who no longer plan to have new pregnancies. Most importantly, HT with the aim of amenorrhea should be tailored for all women following surgery. Continuous use of COCs, or the use of progestogens are simple choices of therapies that result in amenorrhea. The LNG-IUS also appears to be effective in reducing the amount of bleeding (Hurskainen et al., 2004).

In conclusion, endometriosis is a complex inflammatory disease with abundant nerve fibers present in endometriotic tissue. Inflammation contributes independently to pain generation, and also stimulates the growth of nerve fibers in endometriotic lesions. Local estrogen production has a proinflammatory role, as estrogen stimulates the synthesis of prostaglandins (Bulun, 2009). Pain symptoms may respond to hormonal therapies, but in women who do not benefit from their use, surgery is needed. Bowel resection may protect women from recurrences in cases of colorectal involvement. In all women, following operative treatment for RVE, hormonal therapies aimed at amenorrhea should be administered in order to protect them from symptom recurrence. Recurrent symptomatic disease, however, is very uncommon, and most patients greatly benefit from surgical treatment of RVE.

CONCLUSIONS

The following conclusions can be drawn on the basis of the current work:

1. Surgery for RVE results in long-term symptom relief and therefore seems beneficial for those patients in which symptom control cannot be achieved by the use of hormonal treatments.
2. Performing bowel resection, when endometriosis extends to the bowel wall, most likely protects women from symptom recurrence. In addition, all treatments – both hormonal and surgical – that result in the absence of menstrual bleeding significantly reduce the risk of recurrence.
3. Patients with previous endometriosis surgery and intestinal symptoms such as rectal bleeding or pain during defecation are at increased risk of undergoing bowel resection. These patients should be informed of this procedure prior to surgery.
4. Laparoscopy offers a safe approach for RVE surgery, even in cases where colorectal surgery is needed. The rate of complications in laparoscopies does not differ from that seen in laparotomies.
5. Rectovaginal lesions are richly innervated by sympathetic, parasympathetic and sensory nerve fibers. Hormonal therapies reduce the density of these nerve fibers, and this may actually be one of the therapeutic mechanisms of hormonal treatments in RVE.

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